Claims

1.) Method for sequencing a nucleic acid molecule in a thermocycling reaction which initially comprises a nucleic acid molecule, a first primer, a second primer, a reaction buffer, deoxynucleotides or derivatives thereof and at least one dideoxynucleotide or another terminating nucleotide, wherein the thermocycling reaction contains at least one thermostable DNA polymerase with different enzyme activities for incorporating dideoxynucleotides.

2.) Method for sequencing a nucleic acid molecule as claimed in claim 1, wherein the thermocycling reaction contains a first thermostable DNA polymerase and additionally a second thermostable DNA polymerase which has a reduced ability to incorporate dideoxynucleotides in comparison to the said first thermostable DNA polymerase.

3.) Method as claimed in claim 1 or 2, wherein the said first thermostable polymerase has a reduced discrimination against ddNTPs compared to wild-type Taq DNA polymerase in the buffer or under the conditions that are used for the thermocycling.

4.) Method as claimed in one of the claims 1 to 3, wherein the said first thermostable DNA polymerase has a higher processivity than ThermoSequenase and the said second thermostable DNA polymerase has a higher processivity than wild-type Taq DNA polymerase.

5.) Method as claimed in one of the claims 1 to 4, wherein the said first thermostable polymerase is a DNA Taq polymerase with a "Tabor-Richardson" mutation, which also lacks the 5'-3' exonuclease activity, or a functional derivative thereof.

6.) Method as claimed in one of the claims 1 to 5, wherein the said first thermostable polymerase is Taq DNA polymerase (-exo5'-3')(F667Y) or a functional derivative thereof.

7.) Method as claimed in one of the claims 1 to 6, wherein the said second thermostable DNA polymerase is Taq polymerase or a functional derivative thereof.

8.) Method as claimed in one of the claims 1 to 7, wherein the said method is carried out in one step, in a single container, vessel or tube.

9.) Method as claimed in one of the claims 1 to 8, wherein the ratio of the said primers is not equal to 1.

10.) Method as claimed in one of the claims 1 to 9, wherein the ratio of the said primers is about 2:1.

11.) Method as claimed in one of the claims 1 to 10, wherein the said first primer is labelled.

12.) Method as claimed in one of the claims 1 to 11, wherein the said first primer and said second primer are differently labelled.

13.) Method as claimed in one of the claims 1 to 12, wherein the annealing steps of the thermocycling reaction are carried out at a temperature of at least 55°C.

14.) Method as claimed in one of the claims 1 to 13, wherein the thermocycling reaction additionally contains a thermostable pyrophosphatase.

15.) Method as claimed in one of the claims 1 to 14, wherein the said primers have a length of at least 18 nucleotides.

16.) Method as claimed in one of the claims 1 to 15, wherein the said nucleic acid molecule is genomic DNA.

17.) Method as claimed in one of the claims 1 to 16, wherein the said nucleic acid molecule is RNA, the said second polymerase is a thermostable DNA polymerase with reverse transcriptase activity.

18.) Method as claimed in claim 17, wherein the said second polymerase is Tth DNA polymerase or a functional derivative thereof and the reaction is carried out in the presence of $MnCl_2$ or Mn acetate.

19). Method as claimed in one of the claims 1 to 18, wherein the source of the nucleic acid molecules to be sequenced is body fluids such as sperm, urine, blood or blood samples, hairs, single cells or fractions thereof, tissue or fractions thereof, cell cultures, bacteria, viruses or bacteriophages.

20.) Method as claimed in one of the claims 1-19, wherein the thermocycling reaction additionally contains a polymerase-inhibiting agent so that the enzyme activity only occurs at an increased temperature.

21.) Use of the method as claimed in one of the claims 1 to 20 for the determination of a sequence of a nucleic acid.

22.) Use of the method as claimed in one of the claims 1 to 20 for the direct sequencing of eukaryotic genomic DNA.

23.) Use of the method as claimed in one of the claims 1 to 20 for the direct sequencing of human chromosomal or mitochondrial DNA.

24.) Use of the method as claimed in one of the claims 1 to 20 for the direct sequencing of human RNA.

25.) Use of the method as claimed in one of the claims 1 to 20 for the direct sequencing of unpurified plasmid DNA from bacterial colonies.

26.) Use of the method as claimed in one of the claims 1 to 20 for the direct sequencing of unpurified single-stranded or double-stranded DNA from bacteriophages.

27.) Use of the method as claimed in one of the claims 1 to 20 for the detection of genetic mutations or polymorphisms.

28.) Use of the method as claimed in one of the claims 1 to 20 for identifying the origin of the sequenced nucleic.

29.) Use of the method as claimed in one of the claims 1 to 20 for the detection of the presence of foreign or infectious agents in a sample.

30.) Use of the method as claimed in one of the claims 1 to 20 for sequencing a nucleic acid molecule from body fluids such as sperm, urine, blood or blood samples, hairs, single cells or fractions thereof, tissues or fractions thereof, cell cultures, bacteria, viruses or bacteriophages.

31.) Kit for sequencing a nucleic acid molecule containing a reaction buffer, deoxynucleotides or derivatives thereof and at least one dideoxynucleotide or another terminating nucleotide and at least one thermostable DNA polymerase with different abilities to incorporate dideoxynucleotides.

32.) Kit for sequencing a nucleic acid molecule as claimed in claim 31, wherein it contains a first thermostable DNA polymerase and additionally a second thermostable DNA polymerase which, in comparison to the said first thermostable DNA polymerase, has a reduced ability to incorporate dideoxynucleotides.

33.) Kit for sequencing a nucleic acid molecule as claimed in one of the claims 31 or 32, wherein the said first thermostable polymerase is Taq DNA polymerase (-exo5'-3')(F667Y) or a functional derivative thereof and the said second thermostable DNA polymerase is Taq polymerase or a functional derivative thereof.